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Delivering on the promise of gene therapy for hemophilia

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Abstract:

The promise of gene therapy is a single treatment ("one and done") that leads to steady state expression of endogenous factor VIII or factor IX sufficient to achieve a functional cure (free of recurrent hemophilic bleeding) if not normalized hemostasis. The elimination of the need for continued prophylaxis, or factor replacement following trauma or prior to surgery would lead to an annual cost savings. Such optimized health and well-being would be reaching a level of health-equity that was unimaginable several decades ago. "Before anything else, preparation is the key to success." – Alexander Graham Bell. This quote from the famous inventor, scientist and engineer highlights that, although we currently stand on the threshold of this achievement, delivering on this promise will require broad-based multi-stakeholder preparation (scientists, manufacturers, federal regulators, health technology assessors, persons with hemophilia, national advocacy groups, multidisciplinary healthcare teams) with a focused emphasis on *education*, approval of *safe and effective* therapies, removal of barriers to *access* and excellence in *clinical delivery*.

Delivering Education

Following the publication of the nearly complete sequence of the human genome in 2001, Harold Varmus, previously the director of the National Institutes of Health (NIH), wrote an

editorial that remains a call to action for getting ready for gene-based medicine[1]. He highlighted key troubling questions that must be addressed in order for the public to be provided with the full benefits of this revolutionary medical innovation (Figure 1).

As we enter the gene therapy era for hemophilia, a critical limitation is our knowledge and understanding of gene therapy specifically, but also key aspects of the genomic era of medicine. The International Society on Thrombosis and Hemostasis conducted a study of knowledge and perceptions of gene therapy among health care teams and scientists[2]. The results highlighted notable knowledge gaps and educational needs related to gene therapy for hemophilia. Most (66%) of the 5117 survey respondents were physicians and among the 59% of those who were directly involved in the care of patients with hemophilia, 35% indicated that they lacked the ability to explain the science of adeno-associated virus (AAV) gene therapy for hemophilia and 40% indicated limited ability or a lack of comfort in answering patient questions about gene therapy for hemophilia based on the clinical trial results to date. A survey administered by the World Federation of Hemophilia (WFH) to 103 national member organizations (NMOs) and 109 physicians from 76 countries prior to the 1st Gene Therapy Round Table showed that most patients (68%) reported only a basic understanding of gene therapy and 44% of treaters reported only a basic or intermediate understanding[3]. A continuing medical education (CME)-certified clinical practice assessment that measured knowledge, attitudes, and perspectives about gene therapy surveyed 193 physician participants who actively managed patients with hemophilia[4]. This educational activity identified clear deficits about gene therapy and the great majority of healthcare providers lacked confidence in their understanding of gene therapy for hemophilia. These studies have highlighted notable knowledge gaps and education needs related to gene therapy for hemophilia and has informed the development of several important educational initiatives (Table 1). An ISTH educational initiative that launched in 2019 has laid out a roadmap for capacity building for scientists and healthcare providers toward advancing education for the global community. The multidimensional program draws from congress highlights, expert interviews, interactive webinars and the latest updates from clinical resources and publications. The WFH Gene Therapy Round Table series is an annual multi-stakeholder meeting to dialogue on global developments and expected challenges for gene therapy for hemophilia. The WFH, European Haemophilia Consortium and the National Hemophilia Foundation have also partnered with Medscape to deliver CME content intended to bolster knowledge of the science and potential clinical application of gene therapy for hemophilia.

An important aspect of healthcare provider education is equipping them with the knowledge and practical tools to discuss AAV gene therapy with persons with hemophilia (PwH). Two recent manuscripts serve as excellent resources covering key elements of how gene therapy works, who is a suitable candidate, what happens after infusion, what are the expected outcomes, and future considerations[5,6]. These papers cover physician and patient perspectives on efficacy and safety, typical questions that should be addressed before considering gene therapy, and can supplement sources of additional information for healthcare providers and patients from NMOs and scientific societies (Table 1).

Delivering Efficacious and Safe Gene Therapy

Clinical trial design considerations

The platform of current late phase gene therapy for hemophilia uses an ***in vivo*** approach with **non-integrating AAV** vectors to target the **liver**, whereby new genetic material that codes for either FVIII or FIX is **added** to hepatocytes. These clinical trial programs (Table 2) have all had the common goals of determining: if the *in vivo* AAV liver-targeted strategy is safe, what is the ideal dose, how durable is the expression, how predictable are the results and ultimately whether the benefits outweigh the risks. The status of current hemophilia A and B trials have been summarized previously[5,7,8] and share common eligibility and exclusion criteria summarized in Figure 2. The AAV vector is administered as a single intravenous dose, calculated in vector genomes per kg, with subjects enrolled sequentially with escalating doses according to the factor activity achieved. Subjects have typically remained on prophylaxis for several weeks until achieving a factor activity (eg. >5 IU/dL) sufficient to cease prophylaxis. The early phase 1/2 trial results[9-12] have informed the ongoing phase 3 trials wherein subjects have been observed in a 6-month lead-in phase while on traditional factor replacement prophylaxis prior to AAV vector dosing.

Eligibility limitations

Pre-existing immunity

Because AAV is a naturally occurring, non-pathogenic virus, prior exposures are common and an immune response to AAV may be evidenced by anti-AAV antibodies. These antibodies may often be capable of neutralizing the transduction by infused AAV vectors due to cross-reactivity. In nonclinical[13-15] and clinical studies[16], even low titers of pre-existing anti-AAV neutralizing antibodies (NAbs) have been shown to reduce the efficiency of transgene expression. Seroprevalence rates for anti-AAV NAbs can vary by age and geographies. A

European study of 60 healthy donors found an average prevalence rate to AAV8 of 38%[17]. A larger study in 200 European and US donors mapped anti-AAV2, AAV5 and AAV8 immunity, correlating antibodies and cellular responses[18]. This study showed some geographic differences with seroprevalence ranging from 35-74%, but importantly, two thirds of participants were positive for NAbs against more than one serotype. A UK seroprevalence study that recruited patients from seven hemophilia treatment centers identified anti-AAV5 and anti-AA8 antibodies in 21 and 23% of patients, and a neutralizing impact on cellular transduction of 25 and 38%, respectively[19]. In this study, concomitant seropositivity for both AAV5 and AAV8 was also relatively high at 24%. These studies highlight that a considerable proportion of otherwise eligible patients would be deemed ineligible for AAV gene therapy solely on the basis of NAbs. However, the AAV5 vector used in the etranacogene dezaparvovec (AMT-061) clinical trial program showed no correlation of NAbs to AAV5 with FIX activity expression and subjects are currently enrolled in that Phase 3 trial regardless of seropositivity[20].

Inhibitors

A history of an inhibitor to FVIII or FIX has been an exclusion criterion for hemophilia gene therapy in all of the clinical trial programs. This limits another large proportion of patients with hemophilia A in particular, given that factor inhibitors may be seen in >25% of those with severe disease[21-23]. Despite the lack of clinical trial data that will be available, it is reasonable to consider expanded eligibility in future trials or as part of post-marketing evaluation for those who've had transient low-titer inhibitors or a remote history of inhibitor if they are now able to manage their hemophilia with clotting factor concentrates. Preclinical studies[24,25] have suggested that AAV liver-targeted gene therapy for hemophilia could be tolerizing, potentially leading to future clinical trials for those with even active inhibitors.

Pediatric patients

The largely non-integrating AAV vectors are well-suited for liver-directed gene therapy given that, in adults, hepatocytes divide slowly. However, pediatric livers are characterized by hepatocyte proliferation with doublings estimated at age ~2 years and again by school age[26]. Transduction of a pediatric liver would likely lead to a dilutive effect as cell division would not be accompanied by replication of the episomal AAV vector genome and any cellular degradation would then lead to gradual loss of factor expression. With current approaches, retreatment of a previously transduced pediatric patient would not be possible as the seroconversion to AAV would preclude redosing. Accordingly, pediatric patients may be better suited for alternative

approaches such as integrating viral vectors[27,28] or gene editing[29] approaches whereby replication of the vector genome with cell division could sustain stable expression. Given the transition from a high rate of hepatocyte proliferation to a lower rate toward adulthood, adolescents could be a suitable application of current AAV gene therapy strategies and are likely to be included as part of upcoming clinical trial programs or even evaluated in the post-marketing phase.

Efficacy

Efficacious gene therapy should produce factor activity levels sufficient to modify patients with severe disease to a mild or even normal clinical phenotype. These clinical phenotypes have clinical correlates that can in turn be measured by bleeding rates, clotting factor concentrate utilization, and the severity of sequelae such as joint disease and mortality risk.

All the current phase 3 clinical trial programs for hemophilia A and B have been informed by earlier data supporting the stable achievement of factor activity levels sufficient to meet these benchmarks and to significantly impact health-related quality of life measures[9-12]. However, they have also demonstrated an activity discrepancy whereby the one-stage activity assays measure up to 1.6-fold higher than chromogenic assay results for both FVIII and FIX. Contributing to this discrepancy may be transgene optimization strategies (codon optimization, bioengineered FVIII and FIX) or possibly the result of over-expression within hepatocytes[30,31]. The clinical significance of this discrepancy is likely to have the most impact for patients at the extremes of responses, those who achieve low or supraphysiologic factor activity levels, or may influence clinical decision-making around sports participation or the need for exogenous factor for surgical procedures or major trauma. Other important questions that must be elucidated from the phase 3 trials include:

What is the durability of expression? Expression of FVIII and FIX has been demonstrated in multiple preclinical models that has persisted over the life of the animal. The St. Jude/UCL phase 1/2 trial, first reported in 2011, has now demonstrated stable dose-dependent increase in FIX levels in patients with severe hemophilia B following AAV gene therapy that has remained stable at ~5% of normal in the highest dose cohort for >7 years of follow up[7]. Meisbach et al. have reported up to 3 years follow up in severe hemophilia B subjects following an AAV5 vector therapy who have sustained a mean FIX activity of 6.9% in the highest dose cohort[32]. These results with the native FIX transgene could be extrapolated to stable FIX activity levels that are

close to or within the normal range through the inclusion of the hyperactive FIX Padua transgene with 6 to 8-fold higher activity within the current phase 3 clinical trials[11,12]. Pasi et al. have reported multiyear follow up of an AAV5 vector for hemophilia A that has shown durable efficacy with a mean FVIII activity of 33 IU/dL in the 3rd year following transduction[9]. There was an observed decline of the mean FVIII activity from years 1 to 3 that possibly reflects the gradual transition to persistent expression from stable episomal transgenes within nucleated cells. Notably for each of these trials, the persistence of expression was accompanied by sustained reductions in annualized bleed rates as well as >90% reductions in mean annualized use of exogenous clotting factor concentrates. Durability of expression is a critical issue as the expected development of NAbs with AAV liver-directed gene therapy would preclude re-administration of the same vector again without application of some additional innovative strategies. These strategies could include alternating AAV serotypes, direct delivery to the target tissue with avoidance of systemic exposure, use of engineered AAV capsids, and the use of capsid decoys. Recently, plasmapheresis and immunoadsorption techniques show promise with feasibility demonstrated in non-human macaques.[33]

What is the predictability of the response and how much interpatient variability should be expected? The substantially larger number of subjects participating in the phase 3 clinical trials will likely provide new insights into individual biologic variables that may contribute to the predictability and variability of the factor activity levels achieved with any specific gene therapy intervention. Such variability is likely to be most evident for FVIII expression. Variables that should be investigated include factors that influence transduction efficiency and the protein synthetic pathway, interactions with von Willebrand factor (VWF) and determinants of FVIII clearance. Transduction efficiency may be affected by choice of vector and manufacturing processes as there could be variabilities in the AAV receptor characteristics on the hepatocyte or efficiency of formation of stable episomes[34]. The protein synthetic pathway will be affected by variability in mRNA levels and efficiency of protein folding and secretion. VWF levels will influence steady-state FVIII levels and, based on known population variability, could contribute up to 3-fold variation[35]. PwH demonstrate up to 4-fold variation in the half-life of FVIII clotting factor concentrates. This may in large part be due to variability in VWF levels but could also be influenced by variabilities of clearance due to natural polymorphisms in scavenger receptors that are part of FVIII/VWF clearance[36]. Much of this analysis can be conducted with plasma and genomic analyses but will also likely require careful, systematic evaluation of liver biopsy specimens from participants in these trials.

Safety considerations

The safety of AAV gene therapy for hemophilia observed within the multiple phase 1/2 clinical trials has been evaluated sufficiently favorable to justify proceeding with the current phase 3 trials. The much larger numbers of subjects in these trials should allow for a careful weighting of whether the efficacy observed sufficiently outweighs the risks[37]. The risks of AAV liver-directed gene therapy include immediate and short term reactions to the infusion that are expected to be transient and responsive to medical management, rarely requiring any extended observation. Intermediate safety concerns include the impact of supraphysiologic factor activity levels and the self-limited hepatocyte cytotoxicity effects likely driven by both immune and non-immune mechanisms. Although there is no infectious risk from the AAV vectors themselves, it will also be important for AAV gene therapy programs to describe the safety measures necessary during manufacturing to detect, remove, inactivate or prevent the infection of adventitious viruses within the cell lines used for production of the AAV vectors[38,39] Longer term risks include impacts on liver health due to unexpected adverse events or exaggerated cytotoxicity, risks from transduction of non-target tissues outside the liver, as well as the risks from integration events. Integration of transgenic material into the host genome could result in insertional oncogenesis or lead to genetic rearrangements that interrupt, induce or otherwise modify gene structure and/or expression. Although AAV is a "non-integrating" vector, trillions to quadrillions of vector particles are delivered to the patient (with dosing ranges from 1×10^{12} to 6×10^{13} vector genomes per kg) and low level integration (estimated 0.1-1% of transduction events) is known to still occur[37]. This latter risk is the most vexing as the risk of such integration events is not likely to be fully known during any clinical trial observation window that will influence decision-making by the clinical investigative teams or regulators. Evaluation for such longer term risks is the rationale for a global registry specific to gene therapy that would track participants over multiple decades following the clinical trials and commercialization phase of these treatments[3].

Delivering Access to Gene Therapy

If AAV gene therapy demonstrates safety and efficacy within the phase 3 clinical trials, the next most pressing challenge will be regulatory approval, scaling up of manufacturing capacity, health technology assessment and mechanisms of payer reimbursement. Both the European Medicines Agency (EMA) and the Federal Drug Administration (FDA) Center for Biologics Evaluation and Research have provided draft guidance for industry on the development and long term follow up for gene therapy, with the FDA issuing specific guidance on hemophilia gene therapy[40]. The FDA expects to receive 200 investigational new drug applications per year for gene and cell therapies by 2020, and by 2025 expect to approve 10 to 20 such therapies per year[41]. Such demand will require substantial agency budget increases and could be aided by proposed collaboration with the National Institutes of Health to streamline the federal framework and review process with a focus on scientific, safety and ethical issues to attempt to reduce duplication in federal oversight[42].

Manufacturers will be seeking to improve their ability to scale manufacturing to be more efficient through new technologies, expertise and expanded capacity. These may come through acquisitions and strategic partnerships.

The coreHEM project[43] was a multistakeholder initiative to determine a core set of outcome measures required to evaluate efficacy, safety, comparative effectiveness and value of gene therapy for hemophilia with the goal of streamlining regulatory approval, health technology assessment and market access decisions. The coreHEM set of outcome measures have been included within the ongoing phase 3 clinical trials. Notably, the Institute for Clinical and Economic Review (ICER), an independent research organization that objectively evaluates the clinical and economic value of healthcare innovations has announced that it plans to assess the comparative effectiveness and value of valoctocogene roxaparvovec (BMN270) for the treatment of hemophilia compared to FVIII replacement therapy and emicizumab[44].

Such reviews are likely to carry significant influence with payers as they establish their own reimbursement reviews. Delivering access to gene therapy is likely to require innovative payment approaches, even within nationalized health systems given the projected high costs for these therapies. Examples include alternative payment models such as annuity payments that spread the cost over a period of time and payments tied to specific outcome measures (eg. persistence of factor activity, continued bleed control and reduced or eliminated need for factor replacement)[45].

Clinical Delivery of Gene Therapy

This is perhaps the area that will require the most immediate attention within our treatment centers if we are going to be prepared to successfully deliver this potentially curative therapy[46]. Although the gene therapy clinical trials have been conducted within specialized hemophilia treatment centers (HTCs), the HTCS have often had the benefit of their investigational pharmacies, clinical research centers, dedicated research nurses and coordinators. However, even with such institutional supports, many approved clinical trial sites have not been able to successfully navigate the required approvals from their Infection Control Committees, provide the necessary aseptic facilities for reconstitution of the viral vector therapies or identify a suitable clinical infusion site, such that enrolled subjects have often travelled to an experienced dosing site before returning to the home center for the balance of their follow up. The biggest challenge is often education of the professionals in these key areas. This is another opportunity for the HTCs and manufacturers to develop the appropriate training tools and ensure dissemination of the information within the institutional administration and across the clinical staff.

Given these challenges, the ultra-high cost of the therapies, and a single opportunity to achieve the best outcome for the patient, manufacturers and payers will be keenly interested in the training of site staff, monitoring of the performance of the clinical teams reconstituting and administering the infusions as well as the medical monitoring required for managing acute and intermediate term adverse events as described previously. This is likely to drive models of regional/national Centers of Excellence or other clinical delivery partnerships, with an evaluation of "readiness" akin to a site certification[47].

Conclusions

The current replacement therapy era has been marked by a shift from "minimally effective" prophylaxis to regimens that are optimized and even personalized through pharmacokinetic profiling, with an emphasis on more intense prophylaxis and higher trough levels as well as cost-effectiveness[48]. However, biochemistry and genomic advances have ushered in a new era of non-replacement therapy treatments that are meeting remaining unmet needs[49]. These modern innovations have shifted the paradigms of treatment to steady-state prophylaxis rather than the "peaks and troughs" of traditional replacement therapy, can be administered subcutaneously and can function "cross segment", with efficacy in the presence or absence of FVIII/FIX inhibitors and across multiple inherited bleeding disorders. The non-replacement therapies have substantially reduced the burden of prophylaxis with subcutaneous delivery and reduced frequency of administration. The steady-state hemostasis likely

contributes to the excellent efficacy for prophylaxis in pediatric and adult PwH, with and without inhibitors. However, efficacy still requires adherence to a prophylactic regimen and there remains an ongoing annual expense. What has also been sacrificed is a reliable surrogate marker of the hemostatic level achieved, such as has been used for FVIII and FIX monitoring for decades. Gene therapy for hemophilia brings the promise of a single treatment event that would provide steady-state hemostasis at functionally curative, if not normal levels that can be monitored with traditional assays, and PwH liberated from adherence to a prophylaxis regimen and concomitant ongoing reduction in factor utilization with its annual costs (Figure 3). Delivering this promise will require multistakeholder collaboration to evaluate the benefits and risks of this new therapy and well-prepared clinical delivery strategies on a global scale that leverages the best assets of the integrated care model exemplified within the HTCs[50]. Collaborations between NMOs, clinicians, and HTCs on training and education programs will help to build capacity throughout the healthcare delivery systems. Our entire hemophilia community, properly educated and prepared for this next phase of therapy, will be critical in order to facilitate the kind of well-informed shared decision-making that will make delivering on this promise a reality.

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Table 1. Resources for education on gene therapy for hemophilia

Education Source	Title	Content Type	Reference
Haemophilia Journal	How to discuss gene therapy for haemophilia? A patient and physician perspective	• Gene therapy primer • Physician-patient interactions • Risk-benefit discussion	Miesbach et al. (2019)[5]
Blood Reviews	Discussing AAV gene therapy with hemophilia patients: a practical guide	• how gene therapy works • who is a suitable candidate • what happens after infusion, what are the expected outcomes • future considerations	Sidonio et al. (2020)[6]
International Society on Thrombosis and Hemostasis	Gene Therapy in Hemophilia: An ISTH Education Initiative	Multiyear roadmap for capacity building around gene therapy education	genetherapy.isth.org
World Federation of Haemophilia	Gene Therapy for Hemophilia	• Evolution of hemophilia therapy • Basics of gene therapy • Gene therapy for hemophilia	elearning.wfh.org/resource/gene-therapy-for-hemophilia
National Hemophilia Foundation	Future Therapies	• Consumer education • Glossary of terms • Frequently asked questions • Resources	www.hemophilia.org/Bleeding-Disorders/Future-Therapies
European Haemophilia Consortium	EHConversations: Gene Therapy Series	• What is gene therapy? • How does a clinical trial in gene therapy for haemophilia work? • Safety and gene therapy • Gene therapy: A patient's perspective	www.ehc.eu/ehconversations-gene-therapy-series
Medscape	Clinical Advances in Gene Therapy for Hemophilia	• Science of gene therapy • Clinical trial results • Potential clinical application	https://www.medscape.org/sites/advances/gene-therapy-hemophilia
American Society of Gene and Cell Therapy	Education	• Gene therapy 101 • Disease treatments	www.asgct.org/education

Table 2. Current phase 3 clinical trial programs for AAV liver-directed gene therapy for hemophilia A and B.

Name	Clinical Target	AAV Serotype (transgene)	NCT Number (Sponsor)	Phase 1/2 Study References
Valoctocogene roxaparvovec (BMN270)	Hemophilia A	AAV5 (BDD-FVIII)	NCT03370913 (Biomarin)	Pasi et al. (2020)[9]
(SPK-8011)	Hemophilia A	Bioengineered capsid (BDD-FVIII)	NCT03432520 (Spark Therapeutics)	High et al. (2018)[10]
Etranacogene dezaparvovec (AMT-061)	Hemophilia B	AAV5 (FIX Padua)	NCT03569891 (uniQure)	Von Drygalski et al. (2019)[11]
Fidanacogene elparvovec (PF-06838435)	Hemophilia B	Bioengineered capsid (FIX Padua)	NCT03861273 (Pfizer)	George et al. (2017)[12]

BDD, B domain deleted

Figure Legends:

Figure 1. Call to Action for Getting Ready for Gene-Based Medicines[1]

Figure 2. Typical inclusion and exclusion criteria for AAV liver-directed gene therapy for hemophilia. These inclusion and exclusion criteria from the late phase clinical trial programs are likely going to be the same profile of hemophilia patients who will be eligible to receive a commercial AAV liver-targeted gene therapy. Detection of pre-existing immunity to the AAV capsid includes two assays, total antibody and neutralizing antibodies, assessed via transduction inhibition. Neither of these assays are standardized, thus comparisons between laboratories about seroprevalence cannot be accurately made. Determining eligibility for commercial gene therapy will require concomitant approval of a validated assay coincident with the approved AAV gene therapy. Figure courtesy of K. J. Pasi.

Figure 3. New Paradigm of Current and Potential Treatments for Hemophilia.

SQ, subcutaneous

Education Source	Title	Content Type	Reference
Haemophilia Journal	How to discuss gene therapy for haemophilia? A patient and physician perspective	• Gene therapy primer • Physician-patient interactions • Risk-benefit discussion	Miesbach et al. (2019)[5]
Blood Reviews	Discussing AAV gene therapy with hemophilia patients: a practical guide	• how gene therapy works • who is a suitable candidate • what happens after infusion, what are the expected outcomes • future considerations	Sidonio et al. (2020)[6]
International Society on Thrombosis and Hemostasis	Gene Therapy in Hemophilia: An ISTH Education Initiative	Multiyear roadmap for capacity building around gene therapy education	genetherapy.isth.org
World Federation of Haemophilia	Gene Therapy for Hemophilia	• Evolution of hemophilia therapy • Basics of gene therapy • Gene therapy for hemophilia	elearning.wfh.org/resource/gene-therapy-for-hemophilia
National Hemophilia Foundation	Future Therapies	• Consumer education • Glossary of terms • Frequently asked questions • Resources	www.hemophilia.org/Bleeding-Disorders/Future-Therapies
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How can we better train the next generations of physicians to practice genetic medicine?

How can increasingly complex genetic knowledge be made readily accessible to all practitioners when they need it?

How much will the expanded use of gene-based methods further escalate the cost of health care, and who will pay for it?

How can we ensure that these products of our science, largely financed by federal dollars, will reach all the citizens of our country?

- Harold Varmus, 2002

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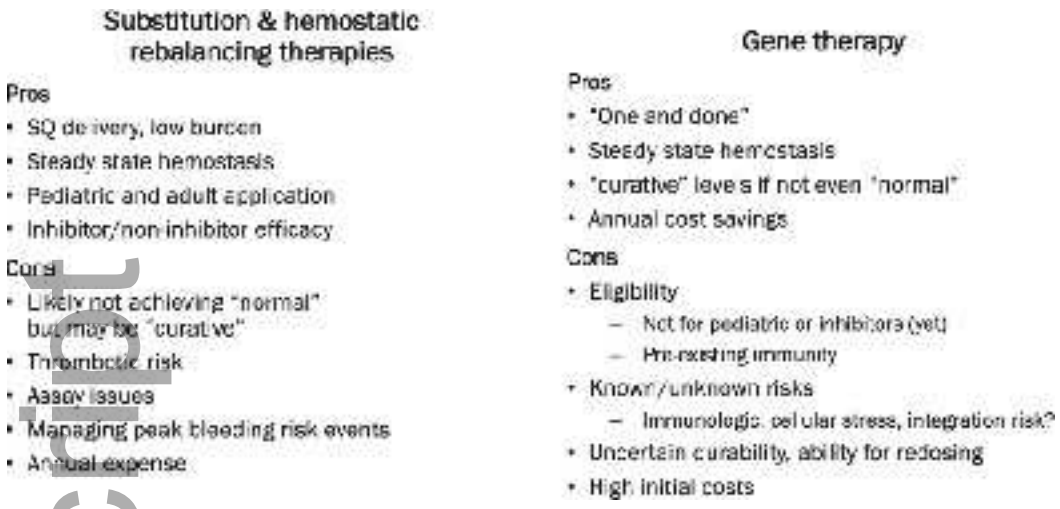

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